# AN HEREDITARY *RICKETTSIA*-LIKE PARASITE OF THE BED BUG (*CIMEX LECTULARIUS*)

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# AN HEREDITARY *RICKETTSIA*-LIKE PARASITE OF THE BED BUG (*CIMEX LECTULARIUS*).

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(*From the Lister Institute of Preventive Medecine.*)

(With Plate II and 1 Text-figure.)

## The Known Species of *Rickettsia*.

The forms under consideration resemble *Rickettsia prowazeki*—the supposed cause of typhus fever—which occurs in lice that have fed on typhus fever patients.

It may therefore not be amiss to describe briefly the organisms which have been grouped by various workers in the same category as *Rickettsia*.

The general characters of "Rickettsia" may be summarised as follows:

(*a*) Bodies of minute size, usually 0·5 $\mu$ in diameter or less, of round or diplococcal shape, though very minute bacillary and even thread-like forms occur.

(*b*) Though resembling very small bacteria in general appearance, they stain much less readily than ordinary bacteria but can be coloured with Giemsa's stain; they are readily decolourised by Gram's method.

(*c*) Absence of motility.

(*d*) Resistance so far, with one exception, to attempts made to cultivate them on artificial media *in vitro*.

(*e*) Their occurrence in very large numbers in the gut and in some cases in other organs of blood-sucking insects.

The known organisms apparently belonging to this group are:

(1) The organism found in the tick—*Dermacentor venustus*—the invertebrate host of the parasite causing Rocky Mountain spotted fever.

This was first described by Ricketts and has since been very thoroughly studied by Wolbach (1919) and called by him *Dermacentroxenus rickettsi*.

It is found in very large numbers in the alimentary canal, salivary glands, muscles and other organs of the tick and is passed on to the next generation of ticks. Similar forms have been described in the blood of men and other animals infected with this disease; Wolbach records its regular occurrence in the tissues of the mammalian host. This organism varies considerably and according to Wolbach, has three distinct morphological forms.

(2) *R. prowazeki* described in detail by da Rocha-Lima (1916) and previously by Ricketts (1909) and Sergent, Foley and Vialatte (1914). This is found in

masses inside the cells of the mid-gut of *Pediculus humanus* after the latter has fed on typhus patients. Although often present in large numbers free in the gut, it appears to multiply inside the epithelial cells. Wolbach and Todd (1920) have described similar forms in the tissues of human sufferers from typhus. The organism varies considerably in shape and size; usually it is round, diplococcal or oval, but often resembles short bacilli; thread forms also occur.

(3) *R. quintana* or *wolhynica*, first described by Toepfer (1916) as the cause of trench fever, occurs in enormous numbers in the lumen of the gut of lice (*P. humanus*) which have fed on trench fever patients. It is more constantly rounded, oval or diplococcal than *R. prowazeki* and is not known to have occurred in thread forms. This species also stains a deeper purple by Giemsa's method. Most writers agree that the organism does not occur inside cells. It may be identical with *R. pediculi*.

(4) *R. pediculi*, first described by Munk and da Rocha-Lima (1917) as an occasional inhabitant of the gut of normal (uninfected) lice and subsequently said by them to be present in trench fever lice. They say that it is indistinguishable from *R. quintana*. It seems probable that the supposedly normal lice in which the parasite has been found had fed upon convalescents from trench fever whose disease had not been diagnosed.

(5) A species of *Rickettsia* found in lice which had fed on persons suffering from "war nephritis" was described by Toepfer (1917), who believed that he could distinguish it from *R. prowazeki* and *R. quintana* by its morphology. This has not been confirmed.

(6) A form of *Rickettsia* which Munk and da Rocha-Lima (*loc. cit.*) found occasionally in a few batches of lice which had either fed on normal persons or on trench fever patients. Munk and da Rocha-Lima stated that the organism was larger and stained more deeply than *R. prowazeki*, that it occurred not only in the lumen of the gut but also inside the cells lining the alimentary canal, in contrast to *R. pediculi* and *R. quintana*. These writers also stated that the organism damaged the cells of the gut and interfered with the powers of the insect to digest blood; they believe it to be a special parasite of the insect and not to be associated with human disease. If Munk and da Rocha-Lima are correct in this opinion their organism resembles the next species and that found in *A. lectularius*, which is described in this paper, so far as restriction to the invertebrate host is concerned.

(7) *R. melophagi*. This organism has been described by Noeller (1917), Sikora (1918), Jungmann (1918), and others, who say it is constantly present in the middle part of the stomach of *Melophagus ovinus* (the sheep "ked"), in large numbers in the older adults, in smaller numbers in young adults, and even occurs in pupae or in very young adults which have not yet sucked blood. The parasite is, they believe, hereditary in the "ked" and not derived from the sheep. The forms described are slightly larger than those of *R. pediculi* or *R. prowazeki*, round, oval or diplococcal in shape. Threads have not been

observed and this organism lies on the surface of the epithelial cells. It has been cultivated on blood agar by Noeller and Jungmann.

(8) *R. ctenocephali* was found by Sikora (*loc. cit.*) in 20 out of 100 cat fleas examined. It is said to be very like *R. quintana*.

(9) The last-named writer found similar forms in smears from the Malpighian tubes of a mouse flea.

### *RICKETTSIA* IN *C. LECTULARIUS*.

Our knowledge of the form we are about to describe, which has hitherto apparently escaped attention, resulted from an attempt to infect bugs with the virus of trench fever by feeding them on patients suffering from this disease. Examinations of smears made from the guts of these insects showed in nearly every case thread-like "bacteria" with some admixture of shorter, rod-like forms (Plate II, fig. 2).

Stained with Giemsa's stain there appeared to be an outer sheath which took the eosin rather lightly while in the interior were granules or groups of granules which stained more deeply and of a purplish hue.

In some cases these smears also showed numbers of small deeply staining coccal or diplococcal bodies which, although slightly larger than *Rickettsia* found in gut and excreta smears of lice that had been fed on trench fever patients, were still sufficiently like them in size and general appearance to suggest that they might be the same species modified by development in the body of an unusual host. The fact that these bodies were not detected in any of the earlier smears from control bugs, although the thread-like forms above described were present, led to unsuccessful attempts to infect two volunteers with the emulsified guts of infected bugs in which the *Rickettsia*-like bodies were present. The examination of further control smears showed, however, that these minute bodies were also present in bugs that had fed only on normal men.

Suspicion of a relation between the rod and thread forms and these minute bodies arose owing to the occurrence of darkly stained granules in the rod and thread forms.

That these minute bodies frequently escaped our notice in smears which showed the long bacterial forms, is due partly to their small size, which makes it difficult to recognise them unless a considerable number are present in a single field; the same difficulty has been found in the case of the Rickettsia of trench fever. The fact that their distribution is often very localised is, however, the chief cause of difficulty in their detection. Whereas the bacillary forms are nearly always generally distributed in smaller or larger numbers in smears of well teased guts or Malpighian tubules, the minute forms, even when present in large numbers, are frequently only to be found in proximity to fragments of the gut or tubules. In some smears they were only seen where they chanced to be escaping from the ruptured end of the gut or a

tubule and no doubt the stage of the parasite in the infected cells affects the readiness with which it is found.

### Motility.

A study of fresh wet preparations by either transmitted light or dark ground illumination failed to reveal any signs of motility.

### Staining.

The most effective staining process tried is the slow method with weak Giemsa stain as generally employed for Rickettsia work, viz. 1 drop of stain to 1 c.c. of distilled water applied for 10–24 hours. All the forms are decolourised by Gram's method; the rod and thread forms are only faintly stained by the fuchsin counter stain. Strong fuchsin produces only a slight effect on the long forms and does not satisfactorily stain the minute coccal or diplococcal bodies. With Giemsa's stain the parasite does not react uniformly, the character of the tissues in its immediate vicinity apparently exercises an influence on the staining process. Where infected cells have been ruptured immediately prior to fixation or if the parasite is included in unbroken cells the long forms stain more intensely than when they are free. In the former situation the stained organism is purple, or if red, shows a gradation to the purple of the internal granules instead of an abrupt transition. It would appear that once the bacillary forms are removed from their natural habitat they rapidly undergo some change which causes them to stain badly.

### Cultivation.

Attempts were made to cultivate the organism on artificial media. All the ordinary media were tried, aerobically and anaerobically, and in addition Dorset's egg medium, Noguchi's medium for spirochaetes, Krumwiede and Pratt's (1913) semi-solid medium which is successful in the cultivation of *B. fusiformis*, and the body juices of a lepidopterous pupa (*Hadena oleracea*), but in each case without a positive result.

In view of the remote possibility of the organism being a stage in the developmental cycle of a spirochaete, some mice were inoculated by scarification and subcutaneous injection with infected Malpighian tubes of the bug but no spirochaetes appeared in the blood.

### Evidence of the hereditary character of the parasite.

In the absence of evidence that the parasite had any second host, theoretical considerations based on the feeding habits of *Cimex lectularius* suggested that the organism must be passed on through the egg. An examination of smears made from the alimentary system of newly hatched unfed bugs showed that the latter were infected. Eggs washed in 2 per cent. lysol for five minutes and then in sterile salt solution contained both rod and thread forms, the

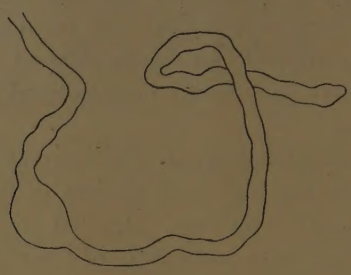
former being more common. This was also the case in smears of unhatched bugs extracted from the egg. Smears of eggs showing the early developmental stage of the bug (as a rule these eggs have to be dissected out of the ovary or made into smears soon after laying), contain immense numbers of more or less well-stained granules, usually poorly defined, but in some cases of distinct *Rickettsia* form and sharp in outline (Plate II, fig. 7); this feature may characterise the greater part of the smear. More frequently, however, it is confined to small patches in what are otherwise areas of ill-defined granules. Since we failed to cultivate the organism we cannot tell whether the ill-defined granules are related to the parasite or whether they are merely particles of protein. The sharply outlined forms are, we believe, undoubtedly a stage in the life-cycle of the organism. The minute bacillary forms that are present among the coccal and diplococcal forms are much more numerous in some smears than in others. Smears of the guts of embryo bugs at a late stage of development extracted from the egg, in some cases show masses of the minute forms deeply stained and clearly defined, lying within the lumen of the gut, where they have presumably been isolated as a part of the detritus of the embryonic process.

In order to obtain additional proof of hereditary infection, ovaries were dissected out of females that had been washed in lysol and then in sterile salt solution. Smears made from eggs extracted from these ovaries usually showed a few threads with some admixture of the shorter bacillary forms in addition to the granular infection alluded to above.

### Intracellular multiplication and development.

Apart from its presence in the undifferentiated egg mass, the development of the parasite seems to be entirely intracellular. No evidence has been obtained of its multiplication in the lumen of the gut or in the body cavity where, however, thread forms have been found. Smears of teased embryos taken from developing eggs and of various organs from older bugs including the ovaries, testes, organ of Berlese and Malpighian tubes show minute coccal, diplococcal and lanceolate forms. These latter are slightly larger than the coccal and diplococcal (*Rickettsia*) forms and stain red instead of purple. Clusters of these red staining forms (Plate II, fig. 1), sometimes accompanied by a few of the *Rickettsia* forms, occur in the cytoplasm. They are best observed, individually, when the infected cell has been ruptured in such a manner as to spread its contents without breaking its nucleus. Very many examples of intermediate forms between these bodies and the longer bacterial forms have been observed; some clusters showed the lanceolate forms together with their various stages of growth into rods (Plate II, fig. 3). The intermediate forms were very often curved. Growth of the rods into threads is shown in almost every smear but apparently only in the enlarged cells of the Malpighian tubes do the threads attain their full length (Plate II, fig. 8).

The minute forms are also to be seen in some of the cells of teased guts in small numbers. In the cells of the seminal pocket on the ventral side of the fourth abdominal segment of adult females, known as the organ of Berlese[1], and in the cells of the Malpighian tubes clusters of the lanceolate forms are of frequent occurrence, sometimes very large ones (Plate II, fig. 4), while the *Rickettsia* forms are frequently present in immense numbers in the enlarged cells of the tubes in company with the thread forms. There would seem, however, to be a distinction between the course of intracellular development in the two organs in that, so far as present observation goes, threads and rods are comparatively scarce in the organ of Berlese, whereas they are exceedingly plentiful in the infected Malpighian tubes, generally in excess of the Rickettsia bodies. The longer bacterial forms are often present in the cells of certain portions of the gut wall, but they are never very numerous in any one cell.



Text-figure. Swelling in Malpighian tube of *Cimex lectularius* due to infection by the parasite. Camera lucida drawing from unmounted dissection of adult female.

Within the cells of the Malpighian tubes the multiplication of the parasites becomes so great that individual cells are swollen to more than twice their normal diameter. This has only been observed in the larger nymphs or adult bugs. It is not uncommon to see two or more tubes each with one or more swellings in the same insect and as the tubes at these points may be two or even three times their normal diameter (Text-figure) the infection is quite apparent under the dissecting microscope.

The enlarged cells in fresh preparations are more transparent than normal ones, sometimes showing a faint greenish hue in the centre of the swelling, and when opened they are found to be packed with the thread forms and granules in varying proportions (Plate II, fig. 8). In sections such cells have a bird's nest appearance owing to the intertwining of the long bacterial

[1] The process of fertilization in the bed bug differs very remarkably from that usual among insects. The penis is inserted into an opening in the right side of the ventral plate of the fourth abdominal segment of the female. Immediately above this opening lies a spherical organ consisting of a mass of cells known as the organ of Berlese. This organ has no duct or outlet and the spermatozoa penetrate through the mass of cells composing the organ into the body cavity, finding their way to the oviducts and fertilizing the eggs *in situ*. The development of the embryo is frequently well advanced before the egg is laid. (For further details see Craig (1915), *Indian Journal of Medical Research*, II. No. 3, pp. 698–705.)

forms (Plate II, fig. 6). Presumably such cells eventually rupture and discharge their contents into either the body cavity or the lumen of the tubule, but this process is not shown in any section yet examined.

### RELATION OF THE *RICKETTSIA* BODIES TO THE THREAD FORMS.

Under dark ground illumination the thread forms have been observed to evacuate granules which, judging by their size, are similar to the darkly stained bodies seen within the thread forms in smear preparations.

Circumstantial evidence suggests that these granules are the same as the Rickettsia bodies which in the first instance attracted our attention and that in bugs which have fed these bodies are largely derived from disruption of thread forms.

### PROBLEM OF THE INFECTION OF THE EGGS.

In addition to their development in certain cells of the alimentary system, thread forms have been found in the blood of the bug taken with a fine capillary pipette from the cut stump of one of the legs of a female and heavy infection of certain cells of the organ of Berlese has been observed in an apparently virgin female (no spermatozoa were observed either in this organ or in a smear of the ovaries which contained no developed eggs). The smear of the ovaries of this female also showed infection, but it was uncertain whether the egg cells were themselves infected. In the case of this apparently virgin female the form found in the sexual organs was chiefly the lanceolate form.

The testes of the males are also infected in some cases but no signs of the parasite were found in smears of the accessory glands of the same insects. As already stated there is no evidence that the organism is motile. It seems possible that the eggs may be infected by the spermatozoa but attempts to obtain evidence from teased preparations that the spermatozoa were infected gave only negative results.

### GENERAL CHARACTER OF THE INFECTION.

Heavy infection with this parasite must be very general, if not universal, among bed bugs. The stock of bugs in which the parasite was first noticed originated from a few specimens taken from an old Essex cottage and as the insects had been confined in a small box and inbred for many generations prior to their use for the trench fever experiments above-mentioned, it is not surprising that almost every bug examined showed one or other of the forms of the parasite. Another race was obtained from an old house situated in Paddington and smears were made from bugs of this stock before breeding from it. Insects of this stock were also found to be generally infected. A further supply was obtained from animal cages in a London laboratory and examined without giving them any chance of further feeding, 19 out of 20 showing the presence of the parasite.

Bugs from two different sources in Warsaw were examined within a day or two of capture and all these were found to be infected.

### Developmental cycle of the parasite.

The scheme set out below is merely tentative, all that is claimed for it is that it is a reasonable explanation of the observed facts and the excuse is offered that by linking up these facts a clearer picture can be conveyed than by the mere recital of the detached details. In the absence of any success in cultivation apart from the host, it is of course only possible to assume a connection between some of the forms in the order in which they are stated.

Starting with the eggs within the ovary, it seems most probable (*a*) that they become infected at the time of fertilisation with the *Rickettsia* form (Plate II, fig. 7), (*b*) that simple multiplication is followed by some of the first generation developing through a bacillary stage into threads while others continue simple multiplication *pari passu* with the presentation of suitable conditions due to the development of the embryo, (*c*) that owing to the massive granule infection of the egg material at this stage, cells of practically every organ of the growing embryo tend to become involved, but only in a certain number are the conditions necessary for intracellular multiplication afforded. In these, clusters of the minute *Rickettsia* forms develop, rapidly changing in favourable situations into the red-staining lanceolate forms through the development of an outer covering or envelope. These enlarge in due course into the long bacillary forms which in their turn release the darkly staining minute *Rickettsia* bodies. The large cells of the Malpighian tubules and their free unencumbered position allow of a much more massive infection than other situations; it is also possible that they afford more stable conditions during the moulting periods and bring the organisms into close proximity to the sexual glands when these are developed, thus increasing the chances of the latter becoming infected. In the course of the very numerous dissections which it has been necessary to make in the progress of this work, it has been noticed how frequently the enlarged cells occur in the distal third of the tubules.

### Suggested name for the parasite.

The organisms known as *Rickettsia* are at present very incompletely described and only recognisable by their more superficial characters, such as morphology and localisation.

On such evidence it is not possible to decide with any certainty how near or remote the phylogenetic relationship of the members of the group to one another may really be, and the present writers therefore consider that Rickettsia should not be accepted as a generic name in the strict sense of the term to cover all the organisms referred to in their opening paragraphs. The smaller group comprising only those concerned with the three mammalian (human) diseases typhus, trench, and Rocky Mountain spotted fevers, would seem to form a more convenient unit.

Nevertheless, since the Rocky Mountain spotted fever organism has been

put into a new genus by Wolbach and on the other hand the form in the sheep "ked" which is not believed, however, to infect the sheep has been called *Rickettsia melophagi* it will be useful to retain *Rickettsia* as a group name for all in the present state of our knowledge. It is in this latter sense that we use it in tentatively naming the above-described parasite of the bed-bug *Rickettsia lectularia*.

## Postscript.

A recent examination of the few specimens of *Cimex hirundinis* which comprise all the bugs of the genus *Cimex*, apart from *C. lectularius* we have been able to obtain up to the present, shows that this species is also parasitised in some of the same organs which are infected in *C. lectularius*. As both the ovaries and testes are involved it is very probable that this will also prove to be an hereditary infection. The organism is, however, much more bacillary in appearance, considerably larger in size and has different staining reactions; in spite of several similarities it is not possible in the present limited state of our knowledge to decide how nearly, if at all, it may be related to the organism described above. We hope to obtain further material to continue our research and should any of our readers be able to obtain living specimens of any of the species within the genus *Cimex*, other than *lectularius*, we shall be very grateful to them if they will send us some.

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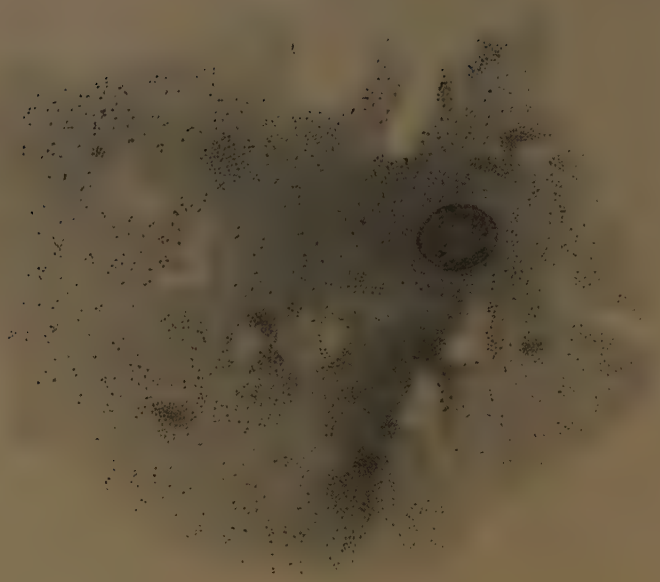
## DESCRIPTION OF PLATE II.

Fig. 1. Minute lanceolate forms issuing from ruptured cell of Malpighian tube ( × 1000).
Fig. 2. Bacterial forms in fragment of gut wall ( × 1300).
Fig. 3. A large cluster of lanceolate forms and developing bacterial forms issuing from ruptured cells of ovary ( × 1000).
Fig. 4. Minute lanceolate forms issuing from ruptured cell of organ of Berlese ( × 1300).
Fig. 5. Thread forms issuing from ruptured end of Malpighian tube ( × 1000).
Fig. 6. Section through Malpighian tube showing mass of thread forms in infected cell ( × 1000).
Fig. 7. Rickettsia and bacillary forms from smears of two eggs. The Rickettsia forms are more heavily stained than is usual in smears of eggs ( × 1300).
Fig. 8. Smear of Malpighian tube showing Rickettsia and thread forms ( × 1300).
Fig. 9. A range of forms selected from smears of various organs ( × 1300).



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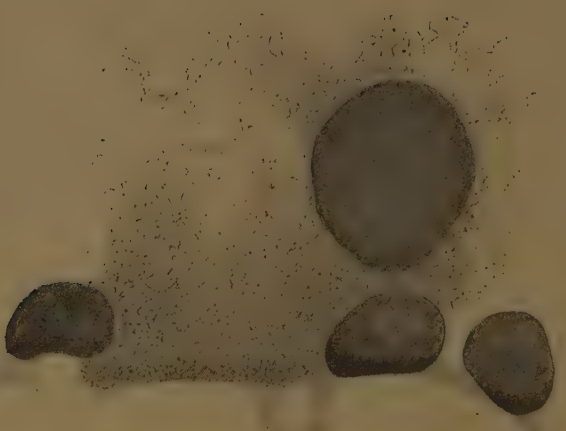

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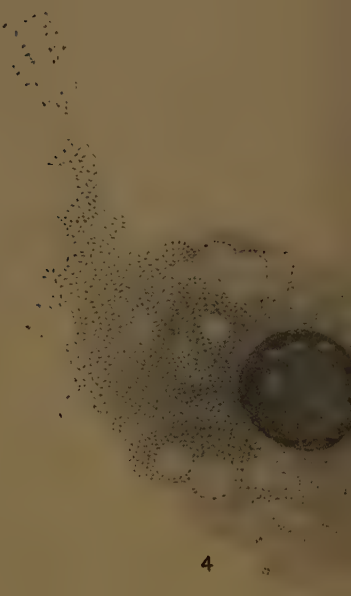

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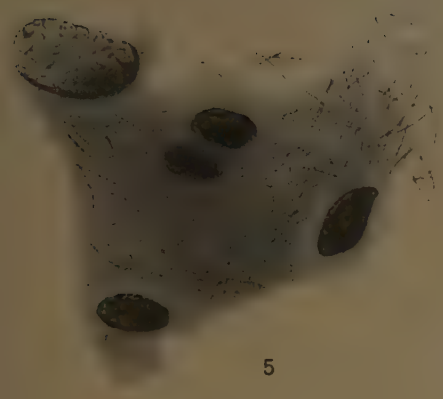

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